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Concise report

Usefulness of serum Krebs von den Lungen-6 for the management of myositis-associated interstitial lung disease

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Abstract

Objective. To identify biomarkers for assessing myositis-associated interstitial lung disease (ILD).

Methods. We reviewed consecutive patients from our institution who had been newly diagnosed with PM, DM, or clinically amyopathic DM during the years 2002–2017. The patients were divided into two groups according to the presence of ILD, and the ILD group was further subdivided into three groups according to the clinical courses of induction failure, relapse and non-relapse. Baseline and time-course changes in the parameters were compared between groups.

Results. Among 110 patients enrolled, 75 (68%) had ILD. Baseline serum Krebs von den Lungen-6 (KL-6) was significantly higher in the ILD group than in the non-ILD group (1120 *vs* 236 U/ml; $P < 0.001$). In the ILD group consisting of the induction failure cases ($n = 3$), the relapse group ($n = 24$) and the non-relapse group ($n = 48$), baseline serum KL-6 was significantly different between the three groups [1971 *vs* 1870 *vs* 935 U/ml, respectively; $P = 0.003$ (relapse group *vs* non-relapse group)]. The time-course changes in serum KL-6 revealed that KL-6 significantly increased along with relapse, with the increase of 625 U/ml relevant to relapse.

Conclusion. Serum KL-6 is a useful biomarker for assessing the disease activity of myositis-associated ILD.

Key words: Krebs von den Lungen-6, interstitial lung disease, myositis, PM, DM, clinically amyopathic DM, relapse, biomarker

CLINICAL SCIENCE

Rheumatology key messages

- Krebs von den Lungen-6 is useful for detecting interstitial lung disease in patients with myositis.
- Krebs von den Lungen-6 predicts and reflects relapse of myositis-associated interstitial lung disease.

Introduction

Idiopathic inflammatory myopathies (IIM), including PM, DM and clinically amyopathic DM (CADM), are a rare group of connective tissue diseases characterized by skeletal muscle inflammation and involvement of other organs such as skin, joints and lungs [1]. Interstitial lung disease (ILD) is a life-threatening major complication, with a prevalence of 20–78% in patients with IIM [2–5]. Although treatment strategies based on solid evidence of myositis-associated ILD have not been established, high-dose glucocorticoids in combination with immunosuppressive agents such as cyclophosphamide and a calcineurin inhibitor are usually administered, and the earlier the introduction of those intensive immunosuppressive treatments the better. However, the response to treatment is heterogeneous, and the high relapse rate of ILD (18–55%) after successful remission induction remains problematic [2–6].

One of the reasons why the management of myositis-associated ILD is so difficult is a lack of effective

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biomarkers. Lactate dehydrogenase (LDH), surfactant protein D, and Krebs von den Lungen-6 (KL-6) are well-known biomarkers for various ILD [7, 8]. However, little is known regarding the usefulness of those biomarkers in ILD associated with myositis. The aim of this study was to identify biomarkers for assessing the disease activity of myositis-associated ILD.

Methods

Patients

We retrospectively reviewed all consecutive patients from our institution who had been newly diagnosed with IIM (including PM, DM and CADM) during the years 2002–2017. Diagnoses of PM and DM were based on Bohan and Peter's criteria [1], and diagnosis of CADM was based on Sontheimer's criteria [9].

We divided patients into two groups according to the presence or absence of radiographically detected ILD. We next divided the ILD group into three subgroups according to the patient's clinical course as follows: those who were dead before achieving remission (induction failure); those who relapsed after remission (relapse group); and those who remained in remission without relapse (non-relapse).

This study was approved by the ethics committee of Keio University School of Medicine (approval number: 20130506). Written informed consent from patients was waived in accordance with the regulations in Japan. All investigations were conducted according to the principles in the Declaration of Helsinki.

Data collection

We collected baseline and follow-up clinical information from patients' medical records: sex, age, diagnosis, blood tests, autoantibodies [anti-aminoacyl tRNA synthetase (anti-ARS) and anti-melanoma differentiation-associated gene 5 (anti-MDA5)] and treatment regimens. Radiological findings on lung X-rays and high-resolution CT (HRCT) and the results of a pulmonary function test were also obtained.

Serum KL-6 was measured using a commercially available ELISA kit (Nanopia KL-6 Reagent, Sekisui Diagnostics, Tokyo, Japan). Anti-ARS and anti-MDA-5 antibodies were detected using a commercially available ELISA kit and/or ribonucleic acid immunoprecipitation, both of which are described elsewhere [10, 11].

Definition

ILD presence was defined by HRCT findings assessed by both a radiologist and a rheumatologist. In the CT scans, the upper lung field (ULF) was defined as the area above the bronchial bifurcation. Induction therapy was defined as treatment provided for ILD within 6 months from the initiation of drug therapy. Relapse of ILD was defined as the fulfilment of new bilateral ground-glass opacities after remission induction observed on HRCT and assessed by both a radiologist and a rheumatologist; no evidence of pulmonary infection; exclusion of other conditions demonstrating ground-glass opacities; requirement of intensified treatment [12].

Statistical analysis

Descriptive values were expressed as median [interquartile range (IQR)]. We conducted Fisher's exact test or the Mann–Whitney *U*-test for univariate analysis. Kaplan–Meier analysis was used to compare the relapse-free survival times. Receiver operating characteristics (ROC) analysis was used to determine the cut-off values of the characteristics. Cox's proportional hazard analysis was used for multivariate analysis with covariates that were identified as potentially significant biomarkers from the previous univariate comparison. Spearman's correlation analysis was used to obtain correlation coefficients. A P-value of <0.05 was regarded as significant. All statistical analyses were performed using the EZR software program (version 1.35, Saitama Medical Center, Jichi Medical University, Saitama, Japan) [13].

Results

Patients and baseline characteristics

One-hundred and forty-seven patients with newly diagnosed IIM were identified. Of these, 37 were excluded because of insufficient information. Of the remaining 110 patients, 75 had ILD (68%, ILD group) and 35 did not (32%, non-ILD group). Of the 75 patients in the ILD group, three died without achieving remission (4%, the induction failure group); 24 relapsed after remission (32%, the relapse group), and 48 remained in remission without relapse (64%, the non-relapse group). A patient flow diagram is shown in Supplementary Fig. S1, available at *Rheumatology* online, and baseline characteristics of the 110 patients enrolled in this study are tabulated in Supplementary Table S1, available at *Rheumatology* online.

ILD group *vs* non-ILD group

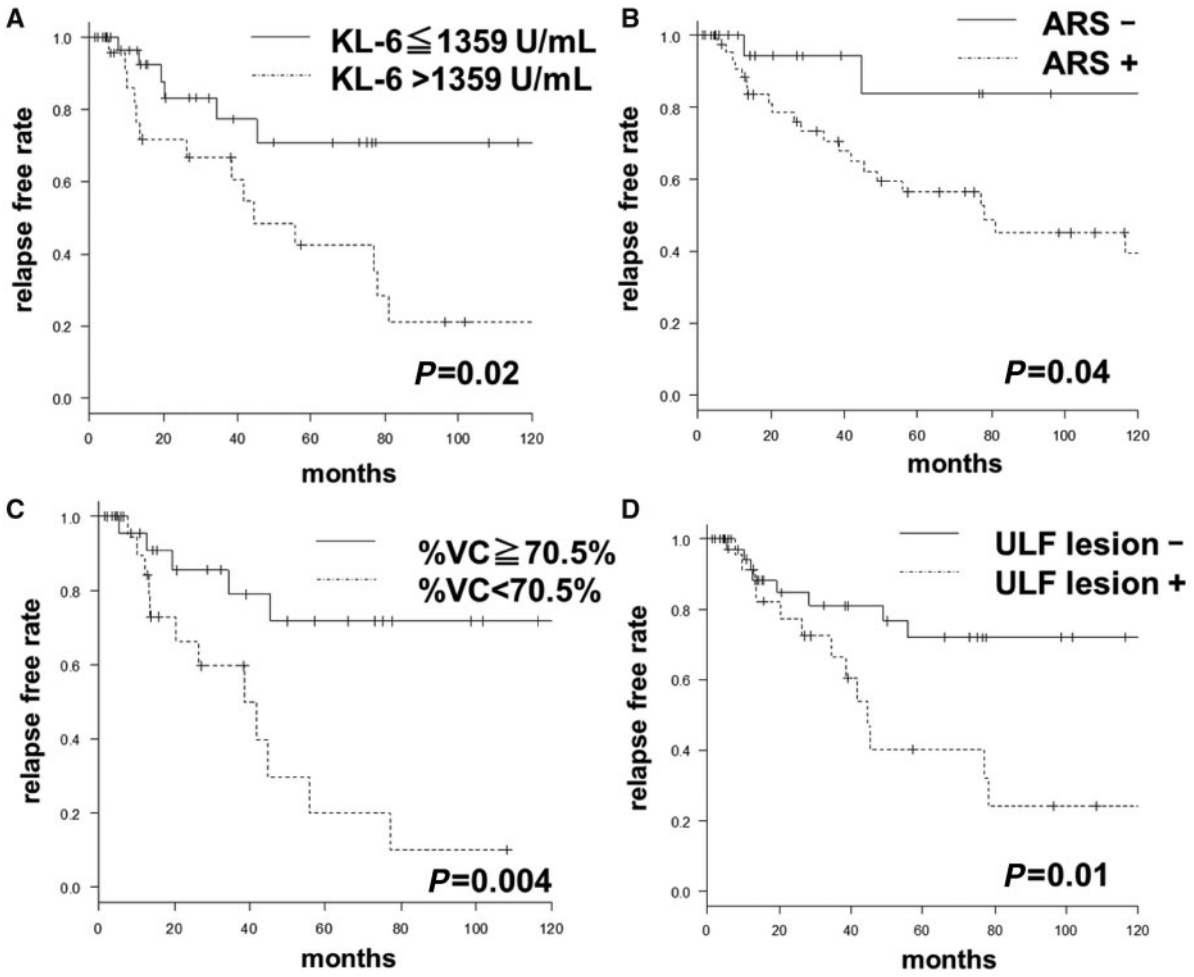
We compared baseline variables between the ILD and non-ILD groups (Supplementary Table S1, available at *Rheumatology* online). The proportion of myositis diagnosis was significantly different between the ILD and the non-ILD groups ($P = 0.02$). The presence of anti-ARS antibodies was more prevalent in the ILD group than in the non-ILD group ($P < 0.001$). Median levels of serum CRP, KL-6 and ferritin were significantly higher in the ILD group than in the non-ILD group (0.95 *vs* 0.05 mg/dl, $P < 0.001$; 1120 *vs* 236 U/ml, $P < 0.001$; 192 *vs* 82 ng/ml, $P = 0.01$, respectively), and serum creatinine kinase was significantly lower in the ILD group compared with the non-ILD group (379 *vs* 968 IU/l, $P = 0.02$). Among those four variables, serum KL-6 was the most reliable for detection of ILD, with an area under the curve of 0.97 and a cut-off of 437 U/ml (sensitivity 87%, specificity 96%; Supplementary Fig. S2, available at *Rheumatology* online). The levels of serum LDH, another marker for ILD, were not significantly different between the two groups (333 *vs* 361 IU/l, $P = 0.67$). In patients with myositis with ILD, serum LDH levels were significantly correlated with serum CK levels ($\rho = 0.72$, $P < 0.001$) but not with serum KL-6 levels ($\rho = -0.12$, $P = 0.29$; Supplementary Fig. S3, available at *Rheumatology* online).

Relapse group *vs* non-relapse group

Among the three subgroups within the ILD group, the induction failure group showed distinct characteristics of high CRP, KL-6 and ferritin levels, although we excluded them from further analyses because this subgroup contained only three patients.

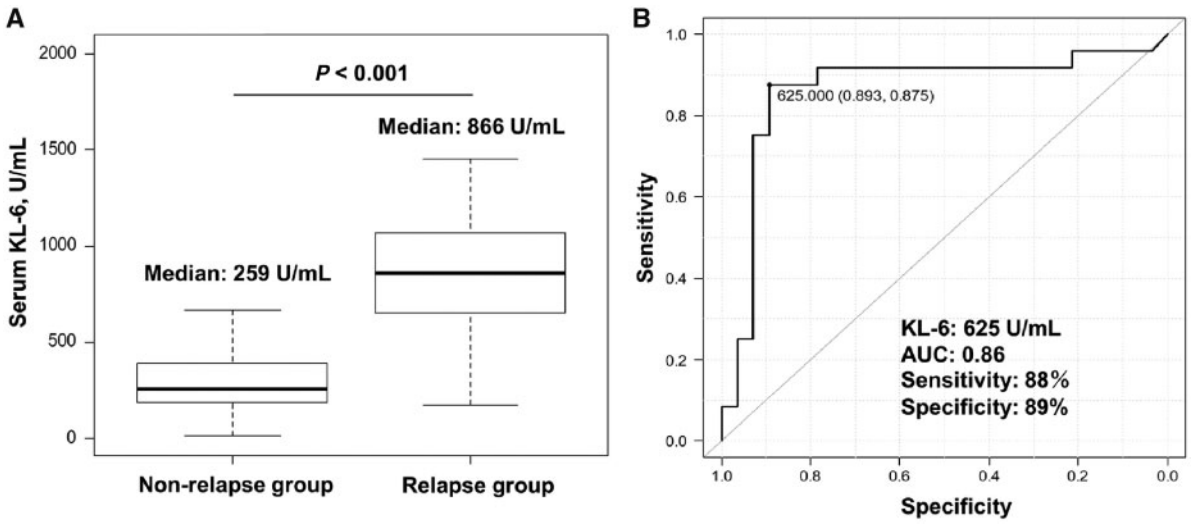
In the comparison of the relapse and the non-relapse groups, no significant differences were found for sex, age and diagnosis between the two groups. Although the initial dose of prednisolone was higher in the relapse group than in the non-relapse group (60 *vs* 50 mg/day, respectively; $P = 0.03$), the following dose of prednisolone (Supplementary Fig. S4, available at *Rheumatology* online) and other immunosuppressive treatment regimens (Supplementary Table S1, available at *Rheumatology* online) were not different between the two groups. Median levels of baseline serum KL-6, the rate of patients who had ULF lesions, and anti-ARS antibody prevalence were significantly higher in the relapse group than in the non-relapse group (1870 *vs* 935 U/ml, $P = 0.003$; 62 *vs* 27%, $P = 0.01$; 88 *vs* 60%, $P = 0.03$, respectively), and median percentage vital capacity (%VC) was significantly lower in the relapse group (66 *vs* 81%, $P = 0.02$). ROC analyses of baseline KL-6 and %VC identified KL-6 of >1359 U/ml and %VC of $<70.5\%$ as significant indicative levels (Supplementary Fig. S5, available at *Rheumatology* online). Kaplan–Meier curves showed that serum KL-6 $>$ 1359 U/ml ($P = 0.02$), %VC $< 70.5\%$ ($P = 0.004$), anti-ARS antibodies ($P = 0.04$), and ULF lesions ($P = 0.01$) were significantly related to the presence of relapse (Fig. 1). Multivariate analysis performed with serum KL-6, %VC, anti-ARS antibodies and ULF lesions revealed that serum KL-6 $>$ 1359 mg/dl was the sole independent risk factor for relapse (hazard ratio = 4.9 (95% CI: 1.0, 24.0), $P < 0.05$).

Fig. 1 Kaplan–Meier analysis for relapse-free rates of myositis-associated ILD



Kaplan–Meier analysis for relapse-free rates of myositis-associated ILD on (**A**) KL-6, (**B**) the presence of ARS, (**C**) %VC and (**D**) ULF lesions. %VC: percentage vital capacity; ARS: aminoacyl tRNA synthetase; ILD: interstitial lung disease; KL-6: Krebs von den Lungen-6; ULF: upper lung field.

Fig. 2 Fluctuation range of serum KL-6 after remission in myositis-associated ILD



(**A**) Fluctuation range of serum KL-6 after remission. (**B**) The cut-off level of KL-6 associated with relapse of myositis-associated ILD by receiver operating characteristic analysis. ILD: interstitial lung disease; KL-6: Krebs von den Lungen-6.

Time-course changes in serum KL-6

For the relapse group, serum KL-6 levels at relapse and the lowest levels during remission were investigated. Six patients who experienced more than one relapse were examined for KL-6 levels during each relapse episode and during each period of remission. For the non-relapse group, the lowest and the highest levels during remission were examined. The time-course change in serum KL-6 in the relapse group showed remarkable decrease during remission and an increase during each relapse episode (Supplementary Fig. 6A, available at *Rheumatology* online). In contrast, serum KL-6 levels in the non-relapse group were stable during those patients' clinical course (Supplementary Fig. 6B, available at *Rheumatology* online). The range of fluctuation in serum KL-6 levels after the first remission induction was significantly wider in the relapse group than in the non-relapse group (866 *vs* 259 U/ml, respectively; $P < 0.001$, Fig. 2A). The increase in serum KL-6 levels of 625 U/ml after remission was identified as a cut-off level for relapse by the ROC analysis (Fig. 2B). Of note, serum LDH levels did not significantly change, with a median increase of only 64 mg/dl (IQR, 52.5–112) in the relapse group.

Discussion

Our study demonstrated that serum KL-6 is a useful biomarker for detecting myositis-associated ILD, predicting relapse and reflecting disease activity in patients with IIM. Interestingly, LDH, another biomarker for idiopathic ILD [8], reflected mainly muscle inflammation and was not correlated with ILD in patients with idiopathic inflammatory myositis. Our study has suggested a useful strategy of using serum KL-6 from the diagnosis to long-time management of myositis-associated ILD.

In our study, ILD was a complication in patients with IIM, and was as frequent as that observed in previous reports [2, 4]. Serum KL-6 efficiently helped distinguish ILD patients from non-ILD patients. In contrast, serum LDH levels were not associated with ILD, but rather with myositis. KL-6 is expressed on the surface membrane of alveolar epithelial cells, while LDH is distributed in various organs and tissues, such as skeletal muscles, lungs, liver and red blood cells. The CK levels were higher in the non-ILD group than in the ILD group in our study, which was consistent with previous reports [3]. Therefore, our study suggested that elevated LDH in patients with myositis-associated ILD is predominantly due to damaged skeletal muscles rather than lungs. Although both KL-6 and LDH are biomarkers for idiopathic ILD [8], KL-6 is a better indicator of myositis-associated ILD.

The high rate of relapse of ILD is a serious problem that has yet to be solved in the management of myositis-associated ILD [2–4]. In previous reports, anti-ARS antibodies and low %VC at diagnosis were reported as risk factors for relapse [3, 4, 14, 15]. We have identified two new risk factors: high serum KL-6 levels and ULF involvement. Taken together, one could say that the combination of anti-ARS antibodies and widely spreading lung lesions is relevant to relapse, assuming KL-6 levels, %VC and upper lung lesions all reflect the extent of the lung injury. It is still unclear whether more intensive treatment could suppress eventual relapse in patients with those risk factors, although maintenance treatment involving glucocorticoid monotherapy has been related with the rate of relapse of ILD [14].

Our study also suggested that serum KL-6 can be used for monitoring disease activity by demonstrating that

serum KL-6 levels were elevated in patients who experienced radiological relapse, while the analogous levels remained stable in patients who did not experience relapse. All relapse cases showed new bilateral ground-glass opacities at the time of relapse most of which coincided with the elevation of KL-6. Of note, in some cases, relapse was recognized by HRCT which was performed because of serum KL-6 elevation detected by periodic KL-6 measurement in outpatient clinics. The increase in serum KL-6 > 625 U/ml during the period of observation was a good indicator for ILD relapse. KL-6 is known to be related to exacerbation of interstitial lung involvement, pulmonary function and poor prognosis of various types of ILD [16–19]. Our study highlighted the importance of serum KL-6 measurement over time in addition to imaging examinations in patients with PM/DM and ILD.

Our study has several limitations. First, this was a retrospective study with a small sample size; this could result in selection bias and confounding, although the number of patients was rather large for such a rare disease. Second, we could not follow the time-course changes in ferritin levels, which are associated with severe ILD [20], because most of our patients lacked sequential ferritin data. Third, the treatment regimen was variable, and this may have affected the incidence of relapse. Our study warrants a prospective study to obtain a consensus regarding the role of using KL-6 in myositis-associated ILD.

In conclusion, serum KL-6 can be a useful biomarker for detection, prediction of eventual relapse and activity monitoring of myositis-associated ILD.

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Supplementary data

Supplementary data are available at *Rheumatology* online.

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